

A METHOD OF REARING DIPLOID DRONES IN A HONEYBEE COLONY*

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Manuscript received for publication 4th January 1969

Summary

Various modifications of techniques for rearing diploid drone brood were tested on 13 900 low-survival larvae (50% female, 50% diploid drone) from inbred queens. Moderate larval survival was obtained after rearing the larvae in an incubator for varying lengths of time and then transferring them to worker cells, but no adult drones emerged. Satisfactory results were obtained from transferring the diploid larvae to drone cells in a colony after 2-3 days in an incubator, and adult drones emerged in relatively greater numbers than from control groups of normal haploid brood. Artificial rearing, however, tended to have an adverse effect on survival rate, and to avoid this, larvae were reared in queen cells of equivalent age in the colony for two days before transferring them to drone cells as before; this method was also successful.

A total of 2286 adult diploid drones were reared in the course of this work, and exact details are given of the techniques recommended.

Introduction

It has been shown that inbred queens of *Apis mellifera* which produce scattered brood in worker cells lay eggs which all hatch (Woyke, 1962); 50% are workers, and 50% are diploid drones (Woyke, 1963a) which are eaten by the workers within a few hours of hatching (Woyke, 1963b). The diploid drone larvae are viable (Woyke, 1963c, 1965b) and can be reared easily outside the colony in an incubator up to the prepupal or pupal stage (Woyke, 1965a). However, since it is very difficult to rear them in an incubator further than this, it was necessary to find an easier method of producing adult diploid drones.

Diploid drone larvae are eaten by the workers if they are in either worker or drone cells (Woyke, 1965c), but not to the same extent if they are in queen cells (Woyke, 1965d); nevertheless, no adult diploid drone was reared in a queen cell because all the larvae fell out of the cells, although one diploid drone was reared in the colony after being previously kept in an incubator for 11 hours (Woyke, 1965c). The facts indicated that there was a possibility of developing a method for successfully rearing adult drones in the colony, and a series of experiments was undertaken.

Methods and Results

From brood produced by sibling-mated queens (see Woyke, 1963a), 13 900 low-survival larvae were hatched in an incubator; about 1600 of these larvae were used for working out a method of rearing diploid drones in the colony, and the rest for testing the new methods. All the larvae were reared in an incubator for some time and then transferred individually to drone, worker or queen cells in the colony. Exact details of the methods finally developed are given at the end of this paper.

* This investigation was supported by a research grant from the United States Department of Agriculture, authorized by Public Law 480, and partly by the Fundação de Amparo à Pesquisa do Estado de São Paulo, Brazil.

About 4000 haploid drone larvae and more than 100 female larvae were used in control or test groups.

Rearing low-survival brood in worker cells

Since normal worker brood develops more satisfactorily in worker cells than in drone cells, and haploid drone larvae are also reared normally in worker cells for the first few days, a comparative study was made of the survival rates of 144 low-survival larvae transferred from the incubator to worker cells in the colony at different ages. Worker larvae of similar age were previously removed from the cells. The number of larvae surviving was checked after 1 and 2 days of rearing in the colony and also when the cells were sealed, at which time the sex of live larvae was determined by the curved capping of cells with drone larvae and the flat capping over worker larvae.

The results are presented in Table 1. An extremely high percentage of low-survival brood survived till the next day in the colony, after the larvae had been previously kept for between 1-2 hours and 3 days on royal jelly in an incubator. Perhaps the fact that relatively low numbers of low-survival larvae were grafted among a large amount of normal brood was partly responsible for the high percentage of survival in most of the series. Ignoring the few larvae used in series 1.2, 39% to 64% of low-survival larvae (both sexes) survived until the time of sealing. In three series 10-35% drones survived, and in series 2.2 more drone than female larvae survived after previously being kept in an incubator for 2 days. The bees started to prepare the convex sealing on the worker cells containing drone larvae, but only isolated cells were sealed, and it seemed as though these cells were too small for the diploid males. No adult diploid drones emerged from worker cells.

This investigation showed that diploid drone larvae which survive the critical period of 1 day or more can be further reared by the bees in the comb at least to the time of sealing.

TABLE 1. Survival rates of low-survival larvae transferred to worker cells in a colony after being hatched and reared for varying times in an incubator.

Series no.	Days in incubator	No. larvae transferred to colony	% survival in colony after:				
			1 day	2 days	Total	5-6 days female	male
1.1	1	18	89	72	44	44	0
1.2	2	5	60	0	0	0	0
1.3	3	20	75	75	45	35	10
2.0	few hr	25	80	72	57	57	0
2.1	1	36	83	69	64	39	25
2.2	2	17	94	94	59	24	35
3.1	1	23	42	42	39	39	0
Total		144					

Rearing low-survival brood in drone cells

The experiments were conducted as in the previous series, but the low-survival larvae were transferred not into worker cells but into drone cells from which the

drone larvae of similar age had been previously removed. Control groups of haploid drones or diploid females reared in the same way were included.

The results are given in Table 2, which shows that relatively low percentages of larvae reared in an incubator for only a few hours survived in the colony until the following day, irrespective of whether they were normal haploids or low-survival larvae. In contrast, a high percentage (more than 80%) of all larvae kept in an incubator for 2 or 3 days survived in the colony for one day, though there were slightly fewer low-survival larvae than controls. The survival rate decreased after 5 days in the colony of the low-survival group and in the control groups.

TABLE 2. Survival rates of larvae transferred to drone cells in a colony after being hatched and reared for varying times in an incubator.

Type of larvae	Days in incubator	No. larvae transferred to colony	% survival in colony after:		
			1 day	2 days	5 days
haploid	few hr	17	65	60	41
low-survival	few hr	50	58	56	6
haploid	2	23	96	90	78
low-survival	2	31	90	90	52
female	2	22	—	86	64
low-survival	2	27	—	63	48
haploid	3	27	89	78	70
low-survival	3	30	83	80	73
female	3	24	92	83	71
low-survival	3	11	82	64	45

The most important result here is that low-survival larvae were reared fairly satisfactorily in drone cells after being kept for 2-3 days in an incubator, but it is also noteworthy that all groups showed increasing mortality with age, indicating that survival was not connected solely with sex or diploidy. It is apparent from these investigations that it is possible to overcome the difficulty of rearing the diploid drones in the colony by isolating the young larvae for a time, because thereafter they are treated by the workers in a similar way to the normal haploid males or diploid females.

Further investigations were therefore confined to the low-survival larvae, in order to find the optimum time for transferring them to the colony. Table 3 gives details of five experimental series in which the larvae were transferred at various ages to the colony from an incubator. The larvae were sexed after 5 days in the colony in series 1, and in series 2.2 and 2.3. In series 4 and 5 the colonies were left undisturbed after the larvae were transferred, until the routine precaution was taken (as in all experiments) of protecting the sealed brood with wire gauze. All the emerging drones were examined genetically, and the size of their testes was recorded. The results of this part of the investigation will be published separately, but it can be stated here that only one drone out of the total of 53 reared was haploid.

Table 3 shows that 14% (52) of the larvae transferred to the colony gave rise to adult diploid drones, and the indication once again was that the least successful age of transfer was a few hours after hatching. Results for other ages were variable,

but in general the best results were obtained when larvae were transferred after 2 or 3 days. However, taking into account the labour involved in rearing the larvae in an incubator, the most efficient time of transfer appears to be after 2 days.

TABLE 3. Survival rates of low-survival larvae transferred to drone cells in a colony after being hatched and reared for varying times in an incubator.

Series no.	Days in incubator	No. larvae transferred to colony	% survival in colony after:					Diploid drones emerging	
			1 day	2 days	5 days			no.	%
					Total	females	males		
1.0	few hr	22	59	36	36	36	0	0	0
1.1	1	7	71	57	57	57	0	0	0
1.2	2	6	0	0	0	0	0	0	0
1.3	3	30	83	80	73	23	50	15	50
1.4	4	5	40	40	20	0	20	0	0
2.0	few hr	50	58	56	6	—	—	1	2
2.1	1	72	53	33	13	—	—	4	6
2.2	2	31	90	90	52	39	13	3	10
2.3	3	11	82	64	45	36	9	1	9
3.0	few hr	25	—	44	44	—	—	0	0
3.2	2	27	—	63	48	—	—	5	19
3.3	3	13	—	38	15	—	—	1	8
4.2	2	24	—	—	—	—	—	5	21
4.3	3	13	—	—	—	—	—	5	38
5.2	2	32	—	—	—	—	—	12	38
Total	few hr	97						1	1
	1	79						4	5
	2	120						25	21
	3	67						22	33
	4	5						0	0
		368						52	14

Comparison of survival rates of low-survival and drone brood reared by the new method

Table 4 gives details of experiments in which the two groups of larvae were reared for 2 days in an incubator and then transferred to drone cells in a colony as before, to check whether diploidy was the factor resulting in poor survival in the colony. The results show that survival rates were variable in both groups, and many larvae were not reared further by the workers in certain experiments, probably indicating unfavourable environmental conditions. In other experiments moderate or high percentages of both groups of brood were sealed. Bearing in mind that low-survival brood consists normally of 50% diploid drones and 50% females (though there was one anomalous result on 10th June 1966), the total percentage of diploid drone brood sealed (30% of the original low-survival larvae) is as good as, or better than, the percentage of normal haploid brood (47%). Diploidy did not therefore appear to exert an adverse effect. In general, more diploid drone larvae were sealed

than worker larvae, presumably because workers are reared less satisfactorily in drone cells.

TABLE 4. Survival rates of low-survival and haploid larvae reared for 2 days in an incubator before being transferred to drone cells in a colony.

Date at start	No. transferred to colony		% survival after 2 days in colony		% brood sealed		
	Low-survival	Haploid	Low-survival	Haploid	Low-survival Diploid male female	Haploid	Haploid
17.8.64	27	23	0	0	0	0	0
17.9.64	43	6	95	83	33	7	66
17.10.64	20	10	85	70	25	50	50
12.7.65	52	13	100	92	60	2	85
11.8.65	28	4	21	25	0	0	0
16.8.65	52	38	2	13	0	0	3
23.8.65	17	17	12	0	0	6	0
10.6.66	44	20	98	60	82	9	60
4.6.67	52	28	44	50	15	8	12
17.6.67	50	57	98	97	46	50	91
24.6.67	59	28	90	100	24	12	82
Total	444	244	65 (287)*	57 (139)	30 (131)	12 (52)	47 (114)

* Figures in brackets are the total numbers in each group.

Survival rates of haploid brood reared throughout larval life under normal conditions, or reared partly in an incubator

An assessment was made of the normal survival rate of haploid drone brood in spring, summer and autumn by counting the larvae 1 day old in drone cells in two combs; one comb was placed in a colony with a queen and the other in a queenless colony, and the surviving drone larvae were counted again after the cells were sealed. Each experiment was repeated three times.

Table 5 shows that a high proportion of larvae were sealed, with the greatest

TABLE 5. Survival rates of haploid drones reared in queenless and queenright colonies at different seasons.

Date and condition	No. larvae 1 day old	No. larvae sealed	% larvae sealed
April-May, with queen	439	353	80
April-May, without queen	368	302	82
June, with queen	606	558	92
June, without queen	381	365	96
Aug.-Sept., with queen	338	237	70
Aug.-Sept., without queen	432	357	85

success in summer, and probably also in the queenless colonies. Comparing these results with those for the haploid larvae in Table 4, the adverse effect of rearing larvae in an incubator for part of their life is seen, although it is noteworthy that, in 3 out of 5 experiments on artificial rearing (Table 4) carried out in the active season (in June or July), the success rates were as high as found here under natural conditions.

Rearing diploid drones from the first day of larval life in the colony

After the demonstration of the rather adverse effects of artificial rearing on survival, experiments were undertaken to work out a method of rearing newly emerged diploid drone larvae in the colony, based on the earlier results (Woyke, 1965*d*) showing that many diploid drone larvae are not eaten by the workers when they are located in queen cells. The difficulty of this method was that no adult drones were reared in queen cells, but since it was now known that only the youngest larvae were normally eaten by the workers, a technique was developed for comparing the earlier method with that described in the present paper.

The low-survival larvae were hatched in the incubator and grafted immediately on to royal jelly in queen cells in the colony, after removal of the queen larvae from them. Three larvae were put in each cell, and when they were 2 days old the larvae were transferred to drone cells from which the haploid larvae had been previously removed (double grafting method). The results are given in Table 6 (series 1a-5a, 6, 7). Control groups of larvae from the same queens were reared for 2 days in an

TABLE 6. Survival rates of low-survival larvae reared from the first day in the colony, compared with control groups reared partly in an incubator. Larvae *Q* were grafted into queen cells, and larvae *D* were transferred to drone cells.

Series no.	Date	No. larvae (<i>Q</i>)	% survival after 2 days	No. larvae (<i>D</i>)	Adult diploid drones reared		
					No.	% (of <i>Q</i>)	% (<i>D</i>)
First two days in queen cells in colony							
1a	20.5	24	54	13	2	8	15
2a	12.6	33	0	0	0	0	0
3a	21.6	45	38	17	6	13	35
4a	27.6	30	67	20	8	27	40
5a	4.7	54	39	21	1	2	5
6	19.6	12	67	8	2	17	25
7	11.10	13	62	8	4	31	50
Total		211	41	87	23	11	26
Control : first day in incubator, second day in queen cells							
8	28.9	43	81	35	4	9	11
Control : first two days in incubator							
1b	20.5	30	97	29	14	47	48
2b	12.6	36	92	33	10	28	30
3b	21.6	43	100	43	5	12	12
4b	27.6	43	100	43	7	16	16
5b	4.7	39	100	39	7	18	18
Total		191	98	187	43	23	23

incubator and then transferred to the same comb (series 1b-5b); in one experiment (series 8) the larvae were reared for the first day in an incubator, for the second day in queen cells in the colony, and subsequently in drone cells.

The mean percentage of larvae surviving after two days in the experimental series was only 41%, compared with 98% in the control series kept in an incubator for both days, and 81% in the control group kept in an incubator for the first day only; the final percentage of the original number of larvae which emerged as adult diploid drones was 11% in the experimental series, 23% in control series 1b-5b and 9% in control series 8. However, if the number of larvae transferred from the queen cells to the drone cells is taken as the basis of calculation, 26% were reared to the adult stage, compared with 23% and 11% in the two control groups. The method could therefore be used to rear adult drones in the colony from the first day of larval life, but in an attempt to increase its efficiency, one factor governing the results of double grafting was next investigated.

Age of queen cells used for grafting

The influence of the age of queen cells (i.e. of the type of brood food deposited there) used for the second grafting in the colony was now studied. Newly hatched female larvae were grafted singly into queen cells from which larvae of different ages had been removed, after the age of each original larva had been recorded on the cork base of the cell. The queen cells were randomized and located in a colony. The number of larvae which survived, the length of sealed queen cells, and the weight of emerging queens, were recorded (Table 7).

TABLE 7. Results of grafting female larvae into queen cells of different ages.

	<i>Age of queen cell (days)</i>		
	1	2	3
No. larvae grafted	20	20	20
No. larvae accepted	15	12	7
Mean length of sealed queen cells (mm)	25	26	27
Mean weight of emerging queens (mg)	201	198	197

Strikingly fewer larvae survived in the queen cells 3 days old than in younger cells, but larvae which did survive developed equally well in queen cells of each age. On the day after the second grafting, the food in the queen cells 1 day old had been replenished by the workers, but in the queen cells 2 and 3 days old it was becoming dry. Only in some cells was a little fresh food placed close to the larvae. (This drying-out of the royal jelly had been observed in earlier experiments where acceptance rates were low—see Table 6). The nurse bees cleaned out the old food before depositing more. Many of the larvae grafted into older queen cells died on the day after grafting, but those that survived were supplied with fresh food by the workers on the second day. Clearly, however, the best results were obtained with queen cells 1 day old, and this has been confirmed using diploid drone larvae.

Rearing diploid drones late in the season

A more serious problem was that the bees did not readily rear any drones (diploid or haploid) late in the season. In 5 experiments in August 191 low-survival larvae were reared for 2 days in an incubator and then grafted into drone cells in queenless

colonies; on the following days the survival rates were, respectively, 75%, 48%, 7%, 0%, 0%. No larvae were sealed. In a control experiment with 23 haploid drones none survived until the next day.

It is difficult to rear diploid drones late in the season when open queen cells are present in the colony, because the bees use all the royal jelly for the queen larvae. Much better results were obtained in the second half of September when the larvae, after being kept in an incubator for 2 days, were transferred to drone cells in a colony with laying workers. Out of 134 low-survival larvae 77% survived until the next day, while 81% of the 16 haploid control larvae survived. The drones were not reared to the adult stage, but 23 diploid larvae were fixed for anatomical and cytological investigations at between 5 and 18 days. In subsequent years many diploid drones were reared late in the season in colonies with laying workers. Here it should be noted that it is helpful to examine the drone cells 2 days after grafting to check whether eggs have been laid in any cells from which grafted larvae have been removed by the workers, since this avoids any difficulty in distinguishing the adult diploid drones.

Some diploid drone larvae and pupae were reared for microscopic investigation even at the end of October and the beginning of November, when the larvae 2 days old were transferred to a colony headed by a drone-laying queen.

To avoid the difficulties associated with the use of queenless colonies, and especially colonies headed by laying workers or drone-laying queens, an experiment was designed to study whether diploid drones can be reared in a colony headed by a normal queen late in the season (towards the end of October). To supply the drone comb with larvae and bee milk, all the worker combs were replaced in two colonies by drone combs, and the colonies fed with syrup and pollen. (Incidentally, this method of obtaining drone cells supplied with bee milk is valuable at any season.) One produced only worker larvae in the drone cells, and the other also had some haploid drone larvae. The former colony was selected for the experiment, in which 38 low-survival larvae hatched in an incubator on 21st October were grafted into queen cells (3 or 4 per cell) in a queenless colony. After two days 25 larvae still survived (66%); 12 of these larvae were fixed and it was found that 7 were female and 5 male; the remaining 13 larvae were grafted into drone cells in the experimental colony. The larvae survived until 7 days old, but the sealing of the cells was not completed. The experiment was repeated on 22nd October with similar results. Thus, though a normal colony unwilling to rear any drone brood of its own will not apparently seal grafted drone larvae, it will rear them up to the time of sealing.

A large-scale test of the new method

The practical value of the new technique—rearing low-survival brood for the first two days of larval life either in an incubator or in queen cells in a colony before transferring it to drone cells in the colony—was mainly tested by undergraduate students who had no previous experience of working with bees. Adult diploid drones were produced from between 5% and 41% of the original low-survival larvae transferred to the colonies in tests carried out between 1964 and 1968 by 11 operators. Altogether 12 718 larvae were transferred and 2164 adult diploid drones reared in these tests; 758 of the adults were reared from 2583 larvae (29%) by the author in Brazil. Unless all the necessary conditions were present, diploid drones were not reared at all, but usually it was not difficult to rear 15–30% diploid drones from the low-survival larvae (50% females), and in some tests the maximum of 50% was achieved. Adding the results of these tests to those quoted earlier in this paper, we can report

a total of 2286 adult diploid drones reared, demonstrating that the new technique is a useful one. The diploid character of these drones was verified by genetical, anatomical, and partly by cytological, examinations.

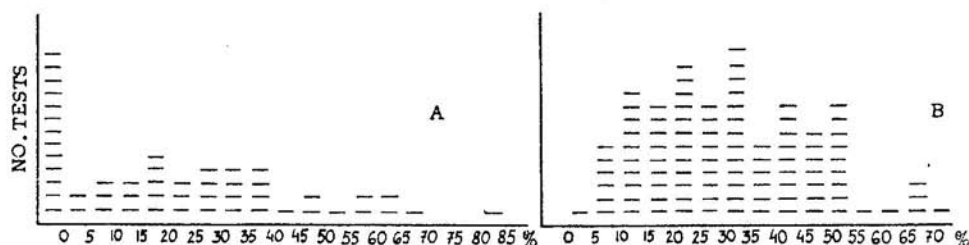


FIG. 1. Percentage of low-survival larvae 2 days old which produced adult diploid drones in various tests (see text).

A - reared by Skowronek 1964-68

B - reared by Woyke in Brazil 1967-68

Within the various series of tests there were widely differing survival rates, as illustrated in Fig. 1; Fig. 1A gives the percentage of adult diploid drones reared by W. Skowronek, a student, and Fig. 1B by the author. The rather surprising occasional occurrence of survival rates above 50% is explained by the small numbers involved here, and consequent departures from the normal 50 : 50 diploid male : female ratio.

Details of the Method for Rearing Adult Diploid Drones in the Colony

First technique: rearing larvae for two days in an incubator before transference to a colony

The main points are as follows:

1. Worker comb with hatching eggs laid by a queen producing low-survival brood is chosen.
2. The comb is wrapped in a moist towel, or inserted in an isolator with water at the bottom, and is placed in an incubator at 34.5°C.
3. Each cell with brood is examined every 3 hours, each newly hatched larva being grafted on to royal jelly (or bee milk) in a queen cup. The royal jelly originates from cells with larvae of similar age to those which are grafted (after removal of the larva). More than 10 larvae can be placed in one queen cup.
4. The queen cups with larvae are placed in an incubator at 34.5°C and a relative humidity of 95-100%.
5. The larvae are transferred every day to new jelly taken from queen cells of the same age.
6. Larvae 2 or 3 days old are transferred to drone cells which had previously contained larvae of similar age reared by the bees; the comb is returned to the colony after the position of each cell with a grafted larva has been accurately noted.
7. The survival rates can be checked 2 days later, and the sex of larvae determined after the cells are capped.
8. The sealed drone cells are protected by wire gauze.

Second technique: rearing in a colony throughout larval life

- 1 and 2. As in first technique.
3. As in first technique, except that only three larvae are placed in one queen cell.
4. Queen cells with the grafted larvae are placed in a rearing colony.
5. Omitted.
- 6, 7, 8. As in first technique.

Acknowledgement

I wish to thank the U.S.D.A. for the financial support, Prof. W. E. Kerr of the University of São Paulo, Brazil, for providing facilities for testing this method in Brazil during the course of studies on bee genetics conducted there, and Dr. M. Delia Seager for help in the preparation of the manuscript.

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